Toxicity and Repellency of Potting Soil Treated with Bifenthrin and Tefluthrin to Red Imported Fire Ants (Hymenopter a: Forinicidae)

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ABSTRACT The LC,So and LC90 of bifenthrin in potting soil for female alates of the red imported fire ant, Solenop.sis invicta Buren, were 1.1 and 5.2 ppm, respectively. For tefluthrin, the LC50 and LC90 were 8.5 and 19.0 ppm, respectively. These results may provide a basis for setting minimum residue levels for the enforcement of quarantine regulations. Pots filled with soil treated with either bifenthrin or tefluthrin at the label rate of 25 ppm were exposed to small colonies of S. invicta without alternative nesting sites. After 48 h, 6% of the bifenthrintreated pots and 24% of the tefluthrin-treated pots were considered infested under quarantine standards. When exposure was lengthened to 2 wk, all pots were uninfested. Implications for the imported fire ant quarantine program are discussed.

KEY WORDS Solenopsis invicta, quarantine, control, soil, nursery, commodity treatment

THE RAPID SPREAD of red imported fire ants, Solenopsis invicta Buren, in the United States has been attributed mainly to the movement of infested nursery stock and sod (Culpepper 1953, Lockley and Collins 1990). Since 1958, a federal quarantine to contain the spread of imported fire ants has been in place. Although quarantine may have slowed the spread of the ants, it has not been completely effective because new infestations have been found outside of quarantined areas. Inexpensive and effective pesticides (e.g., , chlordane) that were incorporated into potting soil for containerized nursery stock were banned in 1979 (Lockley and Collins 1990). By 1990, granular chlolpyrdos was no longer an approved potting soil uarantine treatment because this pesticide bins w;th organic matter, thereby reducing its efficacy (Callcott 1989).

Currently, granular formulations of the pyrethroids bifenthrin and tefluthrin are the only insecticides that are approved by the U.S. Department,, of Agriculture's Animal and Plant Health Inspection Service (APHIS), Plant Pest Quarantine for incorporation into potting soil before planting (APHtS 1995). The,,cost of bifenthrin or tefluthrin soil treatments before planting is several times higher than the formerly approved chlorpyrifos treatmeritl (SNA 1992). To reduce treatment costs, `use of tiered rates was established; this procedure permits reduced. application rates of bifen thrin and tefluthrin to fulfill certification requirements for shorter time periods (APHIS 1995).

The efficacies of granular formulations of bifenthrin and tefluthrin in containerized nursery stock have been based on the mortality of female alates of S. invicta that were confined in treated potting soil. Irk general, efflcaciqus incorporated treatments before planting would cause 100% mortality to S. invicta alates for at least 18 mo..3,4 From these studies, it was difficult to determine concentrations of these insecticides that were lethal to S. invicta when incorporated in soil.

The objectives of this study were to determine the LCS0s and LC90s of bifenthrin and tefluthrin incorporated into potting soil for S. invicta and to examine the deterrence of these concentrations to colony movement into pots containing treated soil. Determination of lethal concentrations would be useful in confirming minimum residues necessary to maintain efficacy and could therefore be used to set minimum residue levels to be detected for quarantine enforcement. In addition, the effectiveness of these pesticides in preventing colony migration into potting soil would be important for nurseries with fire ant colonies on their premises.

Materials and Methods

Our testing method was adapted from procedures used by USDA, Animal and Plant Health

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(3)APHIS internal report. Tefluthrin: a promising new imported fire art quarantine insecticide. November 1992. Imported Fire Ant Station, Gulfport, MS.

(4)APHIS internal report. Bifenthrin: a promising new imported fire ant quarantine insecticide. February 1992, 3rd revised edition. Imported Fire Ant Station, Gulfport, MS.

Inspection Service (USDA-APHIS) Imported Fire Ant Station in Gulfport, MS, and by Banks et al. (1964). A layer (-1 cm) of dental plaster (Castone Dental Stone Type III, Dentsply, York, PA) was added to 2-in. plastic nursery pots (5 cm long, 5 cm wide, 5 cm high) to occlude all drainage holes. This procedure prevented ants from escaping through these holes and the plaster absorbed excess moisture. Potting soil (milled pine bark sphagnum peat moss, and sand [3:1:1 by volume] was air-dried to a bulk density of -~450 g/liter. This soil mix is used by the USDA-APHIS Imported Fire Ant Station to determine the efficacy of insecticides in potting soil against *S. anvicta.*

A granular formulation of either 0.2% bifenthrin (Talstar 0.2 G; FMC, Philadelphia, PA) or 1.5% tefluthrin (Force 1.5 G; Zeneca, Richmond, CA; recently registered as Fireban and distributed by Uniroyal, Middlebury, CT) was mixed individually with 24 g of potting soil per pot. The soil and insecticide mixture was then moistened with 25 ml of water. Female alates of *S. invicta* were separated in the laboratory from field colonies collected in Alachua County, Florida. Alates were held in a tray with moistened paper towels for a maximum of 4 d before each test. In total, 10 female alates were confined in each pot with a petri dish cover. Fumigation within the covered pots was not evident (D.H.O., unpublished data).

After 7 d in the pots, numbers of dead and surviving alates were recorded. Because alates occasionally escaped, only pots that contained ?8 alates were used in the analysis. Bifenthrin concentrations of 0, 0.2, 0.67, 1.0, 1.5, 2.24, 3.34, 5.0, 11.2, and 25 parts per million (ppm) were used in 1941 pots per concentration. For tefluthrin, concentrations of 0, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, and 25.0 ppm were used in 15-35 pots per concentration. For each concentration, an average percentage mortality was determined from 2-6 replicates (4-10 pots per replicate). Replicates consisted of pots containing soil with similar bulk densities and alates that had been collected from the same colony. Pots were held indoors at ambient room temperatures ranging from 22.8 to 29.1°C.

Regression analysis (PROC REG; SAS Institute 1988) was used to estimate LC50 and LC90 values for each insecticide. Percentage mortalities were adjusted usir* Abbott formula (Abbott 1925), and concentrations were logarithmically transformed. The treatment level of 25 ppm was excluded from the bifenthrin analysis because. the percentage mortality was similar to the 11.2 ppm level.

The repellencies of the LC50, LC90 (estimated from data from the previously described experiment), and maximum label rate (25 ppm) of the bifenthrin and tefluthrin were examined by exposing treated potting soil to *S.'invicta* colonies. Soil was treated as described in the lethal concentration studies, except that pots did not have a plaster layer. Thus, ants had access to the soil through the drainage holes. Average ± SD dry bulk densities ranged from 487.7 ± 29.9 to 622.6 ± 15.6 g/liter for each replicate. Treated pots were held overnight, then were placed in open plastic boxes (33 cm long, 18.4 cm wide, 10.8 cm high) that contained small *S. invicta* colonies of =750 workers, 1.2 ml (1/4 teaspoon) of brood, and 1 queen. Fluon (ICI Americas, Wilmington, DE) was applied to the inner sides of the boxes to prevent ants from escaping. Pots were held and exposed indoors at ambient room temperatures ranging from 21.128.9°C. Pots were examined for the presence of ants after 48 h.

Pots with live adult ants and brood within the soil, clinging to the exterior sides of the pot, or under the pot were considered to be infested. In addition, pots with virtually all of the worker ants only (i.e., excluding brood or queens) within the soil, or on or under the pots also were considered to be infested. Pots that did not have brood or the

Tjeen, and at most only a few workers in or on e pot, were considered to be uninfested. To indicate the degree of infestation, the number of ants observed on the inner sides of a pot and the surface of the soil were counted.

When most of a colony moved into the soil, counts were obtained by subtracting the number of ants on the outside of the pot from the original 750 ants. Pots were not emptied so that ant counts simulated quarantine inspections. The average per= centages of uninfested pots were compared among treatments for each compound by analysis of variance (ANOVA) and Tukey honestly significant difference (HSD) test (PROC ANOVA; SAS Institute 1988). Averages were based on 5 replicates of 10 pots per treatment. .

To examine the effect of exposure length on repellency, 10 pots per concentration were treated, exposed to colonies, and examined for infestation at 48 h, 1-4 wk, and at 7 wk by using the methods of the preceding study. Once a week, pots were moistened (without any runoff) with 5-15 ml of water. Colonies also were provided water, crickets, and honeywater. Average ± SD dry bulk density of the potting soil was 459.3 -"_- 5.0 goiter, and ambient indoor temperatures ranged from 22.2 to 27.8°C during the study. To determine if exposed colonies could still infest pots over the extended exposure period, 10 colonies also were held without any pots until the final sample, when untreated pots were placed with these colonies and in any boxes containing uninfested treated pots. After 72 h, untreated pots were examined for infestation and the number of live adult ants were counted from all treatments. The percentage of infested pots was determined for each treatment. An ANOVA and Tukey HSD test (PROC ANOVA; SAS Institute 1988) were used to compare the number of ants among treatments.

Results and Discussion

LC9os were 48 and 24% lower than the minimum label rate of 10 ppm for bifenthrin and the

Table 1. Concentrations (ppm) lethal to S. invicta of bifenthrin (Talstar 0.2 G) and tefluthrin (Force 1.5 G) in potting soil

Active ingredient	n°	slope (= SE)	LCsob (95% CL)	LC9ob (95% CL)	-2
Bifenthrin	252	0.85 (0.096)	1.09 (0.73-1.53)	5.17 (3.85- 6.85)	0.7423
Tefluthrin	211	1.23 (0.119)	8.47 (7.41- 9.66)	19.01 (15.71-22.96)	0.8106

° Total number of pots including controls. Slope and lethal concentrations were based on mean percentage mortalities of 2-6 replicates per concentration of active ingredient. Each replicate contained 4-10 pots.

b Lethal concentrations were obtained from the following linear regression models for bifenthrin; y = 0.2277 + 0.8507x; and tefluthrin: y = -0.7020 + 1.2312x; where y is proportional mortality and x is loglo(concentration + 1 [in ppm]). Reported lethal concentrations were back-transformed.

rate of 25 ppm for tefluthrin, respectively (Table 1). The amount of bifenthrin needed to achieve 99.9986% mortality was estimated to be 7.1 ppm (5.1-9.8 ppm 95% CL), which is below the minimum tiered rate of 10 ppm. For tefluthrin, 23.1 ppm (18.6-28.8 ppm 95% CL) was required for the same level of mortality. An APHIS report3 provided **LC90s** for bifenthrin against S. invicta alate queens that ranged from 11.62 ppm in a commercial potting soil (Strong-Lite, Pinebluff, AR) to 0.99 ppm in river sand. In the standard **Gulfport potting soil, a LC9o** of 3.73 ppm was reported. These results suggest varying toxicities among potting soil types. In addition, **LC90s** for major workers held in the commercial mix and river sand were 68 (3.70 ppm) and 94% (0.057 ppm) lower than those for alates, respectively. Thus, their alate lethal concentration values were more conservative. However, all of the above results were preliminary and variability within kbe:tests was high3 (APHIS). Our lethal concentration values were higher and could serve as a conservative estimate of the minimum concentration that should be detected in residue analyses , of potting soils that are checked for quarantine compliance.

The repellency of treated potting soil to the movement of fire ant colonies into pots increased with the higher concentrations. As the treatment rates increased the number of uninfested pots increased ;(Fig. 1). **LC9o** and 25 ppm rates of both compounds yielded significantly less infested pots than the lower rate (Table 2). At 25 ppm, colonies were located only'on the exterior of the infested pots (Fig. 1). .

Of the pots treated with bifenthrin and considered uninfested, 9.6 i- 13.0 (± SD) worker ants per pot were counted in 64% (54 of 85) of the pots. For the uninfested, tefluthrin-treated pots, 23.6 -!41.1 ants were counted from 52% (40 of 77) of these pots. As application rates increased for both formulations, the average number of ants decreased (Table 3). At the maximum label rate of 25 ppm for both treatments, worker ants were still

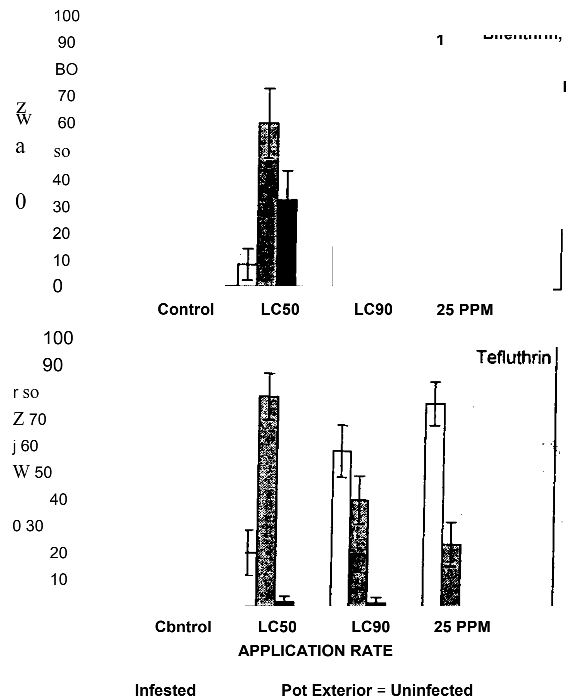

Fig. 1. Percentage of pots containing soil treated with bifenthrin or tefluthrin that were infested, had colonies on the exterior surface of pots, or were uninfected after exposure to S. inoicta colonies for 48 h and without alternative nest sites.

observed in 56% (56 of 100) of the treated pots. Thus, the recommended application rate of 25 ppm for containerized plants was not a complete deterrence to worker ant movement onto potting soil under laboratory conditions after 48 h. Under natural conditions, alternative nesting sites should be available and may reduce the probability of colony infestation.

Increased exposure time to treated soil reduced infestations by causing mortality or movement from treated areas (Table 4). At 25 ppm, only I colony was found temporarily on the exterior of 1 pot after 2 wk of exposure. S. invicta colonies fre-

Table 2. Percentages of pots treated with bifenthrin (Talstar 0.2 G) or tefluthrin (Force 1.5 G) that were infested by S. invicta colonies after a 48-h exposure without alternative nest sites

	Treatment rate			
	Control	LCgo°	LCgob	Label rate°
Bifenthrin	100 ± O.Oa	92 ± 5.8a	32 ± 5.8b	6 ± 6.Oc
Tefluthrin	100 ± O.Oa	80 = 8.4a	42 ± 9.76	24 ± 8.16

Values are means ± SE. Within a row, means followed by the same letter are not significantly different ($P > 0.05$; Tukey HSD test [SAS Institute 1988]). Means based on 5 replicates of 10 pots per replicate for each treatment rate. ° Bifenthrin, 1.1 ppm; tefluthrin, 8.5 ppm. b Bifenthrin, 5.2 ppm; tefluthrin, 19.0 ppm. Label rate, 25 ppm.

Table 3. Mean number of ants in pots designated as being uninfested that were treated with bifenthrin (Talstar 0.2 G) or tefluthrin (Force 1.5 G)

	Treatment rate		
	LC_{50}[a]	LC9o6	Label rate'
Bifenthrin	20.8	12.8	3.2
(~ SD) n	(19.9)	(14.8)	(1.9)
Tefluthrin	4	29	21
(= SD) n	64.5	30.2	10.1
	(72.8)	(49.0)	(12.1)
	4	16	20

All control pots were infested. a Bifenthrin, 1.1 ppm; tefluthrin, 8.5 ppm. 6 Bifenthrin, 5.2 ppm; tefluthrin, 19.0 ppm. c Label rate, 25 ppm. d Number of uninfested pots.

quendy move away from nest sites treated with insecticides (Williams and Lofgren 1983). However, for the lower concentrations, at least 20% of the pots were infested. Throughout the study, colonies were found on the exterior sides of pots treated with 19 ppm (LC90) of tefluthrin, which is 90 and 27% higher than the recommended tiered rates of 10 and 15 ppm for 6- and 12-mo certification periods, respectively.

At the maximum rate of 25 ppm, the incorporation of granular bifenthrin or tefluthrin in potting soil allows containerized nursery stock to be eligible for certification for shipment continuously (APHIS 1995). At 25 ppm, we initially observed ants clinging on the exterior of some pots but did not observe colony movement into the soil (Fig. 1). When soil treated with 25 ppm of either compound was exposed to colonies for >1 wk, only 1 pot temporarily had a colony on its exterior. Although colonies on the exterior of pots would be grounds for rejection of shipments, the lack of alternative nest sites in our laboratory tests probably represent more rigorous circumstances than would be found under most field conditions. Therefore, the probability of infestations or shipment rejection because of the presence of colonies on or in pots filled with soil treated at the maximum label rate of 25 ppm should be minimal. Nonetheless, our results suggest that infestations may be more prevalent in soil treated at the lower tiered rates of tefluthrin. Further research is needed to establish the effect of altemative nesting sites under field conditions on colony movement into treated potting soil.

Although recommendations for containerized soil treatment are based on the elimination of newly mated queens, they are just one facet of the quarantine program. Nurseries in voluntary compliance with the "Fire Ant Free Nursery Program" must eliminate fire ant colonies on premises in addition to treating potting soil to ship containerized nursery stock outside the quarantine area without inspection (APHIS 1985, 1995). Similar approaches of reducing natural pest populations before postharvest treatments have eliminated quarantine pests in floriculture and fruit crops (Hata et al. 1992, Jang and Moffitt 1994). For imported fire ants, a program that reduces both the sources of infestation and potential harborages, such as inadequately treated potting soil, should be more effective in minimizing the infestation of nursery stock.

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Table 4. Percentage of infested pots nudzber of S. invicta from infested pots per treatment' of hifenthrin (Talstar 0.2 G) or tefluthrin (Force 1.5 G)

Weeks	Ctrlb	Bifenthrin			Tefluthrin		
		LCsd~ ~	LCgpd	Label,	LC50	LCyp	Label
0.3f	100	90	80	10	50	50	20
1	100	90	80	10	70	80	20
2	100	100	100	0	50	50	0
3	100	100	90	0	40	40	10
4	100	100	90	0	50	40	0
7	90	100	60	0	40	20	0
Antsy	865a	598a	17b	Ob	108b	756	O6
± SD	(309)	(249)	(35)	-	(233)	(159)	-

n = 10 pots per concentration. 6 Control. 6 Bifenthrin, 1.1 ppm; tefluthrin, 8.5 ppm. d LCgp, bifenthrin = 5.2 ppm; tefluthrin = 19.0 ppm. e Label rate, 25 ppm. f0.3wk=2d. B Number of ants (mean ± SD) from week 7 only. Means followed by the same letter are not significantly different (P > 0.05) by Tukey HSD test on square root transformed data (SAS Institute 1988). Untransformed means are presented.

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